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Effects of deuterium oxide on mechano-sensory receptor

(crayfish receptor organ/receptor potential)

SHIGEHIRO NAKAJIMA AND TOSHIKO KURODA

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

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ABSTRACT **In the crayfish stretch receptor organ, a total substitution of D_2O for H_2O in the bathing solution produced a decrease in the amplitude of the receptor potential to a level of 34% of the control. The electrical resistance of the receptor neuron was slightly increased by the D_2O substitution. The input capacitance of the neuron was unchanged. The viscoelastic properties of the receptor muscle were not altered by D_2O. Thus, we conclude that D_2O has an inhibitory effect on the transduction process which links the deformation of the dendrites with the permeability increase that is responsible for the receptor potential.**

Deuterium is known to have a variety of effects on various kinds of living systems (1). Of particular interest are the following effects of deuterium at the cellular or organismic level: (*i*) a wide variety of biological rhythms such as the circadian rhythm or the discharge rate of electric organ of electric fish are affected (2–5), (*ii*) the time course of nerve action potentials is slowed down (6, 7), and (*iii*) the calcium release from the sarcoplasmic reticulum of muscle is inhibited (8–11). In this preliminary communication, we report that deuterium oxide has a fairly large inhibitory effect on the receptor potential mechanism of the crayfish stretch receptor. Before we found this deuterium effect, we tried various kinds of drugs and chemicals, none of which had a marked effect on the receptor potential.

Slowly adapting stretch receptor organs, isolated from the second abdominal segment of crayfish (*Orconectes*), were used. The method of recording the receptor potentials from the receptor neuron through an intracellular microelectrode, and the technique of stretching the receptor muscle were similar to the ones used previously (12). The receptor organ was mounted in an experimental chamber, through which bathing solutions were perfused continuously. The standard fluid was the original Harreveld solution (13) with 2.4 mM $NaHCO_3$ (pH 7.7–7.8). The D_2O/Harreveld solution was made by dissolving the same amount of salts in D_2O as found in the Harreveld solution (the D_2O was redistilled from 99.7% D_2O, New England Nuclear).

Stretches of the receptor muscle in the normal Harreveld solution evoke the receptor potentials (the generator potentials), the amplitude of which varies according to the magnitude of the stretch. If the receptor potential exceeds a threshold, then repetitive all-or-nothing action potentials are produced (14, 15), as is shown in Fig. 1A. Application of tetrodotoxin (TTX) abolishes the action potentials, but leaves the receptor potentials intact (16) (Fig. 1B).

When the normal Harreveld solution was replaced by the D_2O/Harreveld solution, the amplitude of the receptor potential was considerably reduced. An example is shown in Fig. 1C. In experiments on five receptors, the amplitude of the receptor potential (measured at 1 sec after the start of the stretch) decreased on the average to 34% (ranging 25–47%) of the control value. The effect of D_2O was reversible as shown in Fig. 1D.

Abbreviation: TTX, tetrodotoxin.

The time course of the receptor potential in D_2O seems to be slightly different from that in H_2O. The difference would become apparent, if one compares record B with record C after the peak heights of the receptor potentials are normalized. Although the peak of the receptor potential is attained almost at the same time, the following rapid decline (adaptation) is more rapid in D_2O than in H_2O. This is probably caused by a nonlinear relation between the magnitude of the receptor potential and the conductance increase that is responsible for the receptor potential. When the receptor potential becomes large, as in B, then by approaching the reversal potential, a given increment of the conductance increase will result in a smaller increment of the receptor potential. Another factor would be that, when depolarization is large, the delayed rectification (17, 18) occurs, which results in apparently small receptor potentials. If we consider these factors, then it seems that the main effect of D_2O is to reduce the magnitude of the conductance increase that is responsible for the receptor potential without large effects on its time course.

The decrease of the receptor potential by D_2O is not due to a decrease in the passive membrane resistance. In the experiments shown in Fig. 1, the input resistance of the neuron, measured by injecting square-wave currents through a bridge circuit, was 4.7 MΩ in the first control (B), 3.5 MΩ in the D_2O solution (C), and 3.5 MΩ in the second control (D). In the five experiments performed, the resistance in D_2O averaged 103% (range, 85–150%) of the control values. The total electrical capacity of the neuron was not altered by D_2O.

The pD of D_2O solution was 8.2–8.4, by assuming that the pD = reading in pH meter + 0.4 (see ref. 19); this value is higher than the pH value of normal Harreveld solution. In two cells, we compared the receptor potentials in a D_2O solution with pD = 8.3, and those in a D_2O solution with pD = 7.9; the change of pD was achieved by reducing $NaHCO_3$. The amplitude of the receptor potentials in these two solutions was practically the same.

We suspected that the reduction of the receptor potential in D_2O might be caused by possible changes in the visco-elastic property of the receptor muscle. Thus, the stress-strain relationship of the receptor muscle was investigated by using a method similar to the one used previously (12). It was revealed that D_2O did not have measurable effects on the visco-elastic property of the muscle. However, this experiment does not exclude the possibility that the elasticity of the connective tissue which couples the dendrite tips with muscle is slightly altered.

The amplitude of the receptor potential is not a good indicator to quantify the transduction mechanism of the receptor potential. Instead, the increase in conductance (ΔG) that occurs upon the stretch should be evaluated. We calculated ΔG for each record of the receptor potential, by using the value of membrane resistance, and by taking into account the nonlinear relationship between ΔG and the amplitude of the receptor potential (20). The reversal point of the receptor potential was

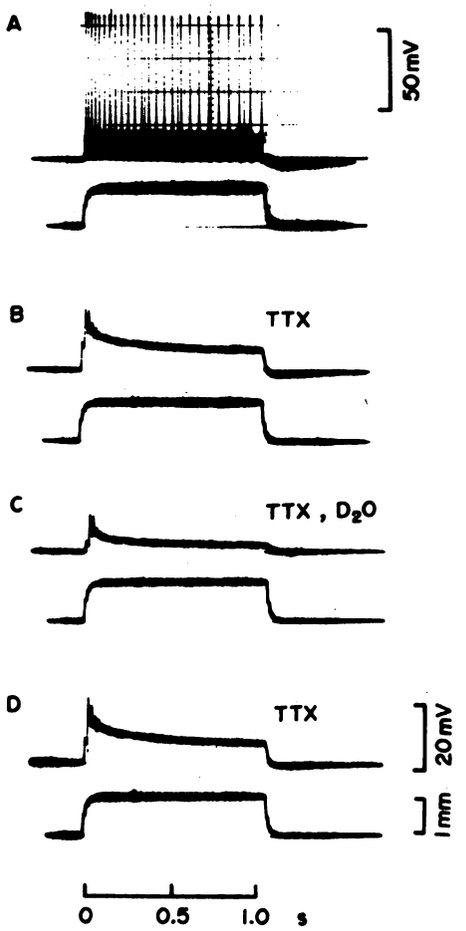

FIG. 1. Membrane potential changes recorded from a receptor neuron by an intracellular microelectrode. The potential changes are in the upper beams, and the stretches of the receptor organ (i.e., the sum of the displacements of both ends of the receptor muscle) are in the lower beams. (A) Action potentials evoked by a stretch in the normal Harreveld solution. (B–D) Receptor potentials after application of TTX (0.1 μg/ml). In (B), a control receptor potential was recorded in H_2O/Harreveld solution with tetrodotoxin. In (C), a receptor potential was recorded after a complete exchange of the solution with the D_2O/Harreveld. The chamber was perfused for 7 min with D_2O/Harreveld having 14 times the chamber volume (2.5 ml). (D) After a complete replacement of D_2O with H_2O/Harreveld. Perfusion volume was 28 times the chamber volume. Perfusion time was 18 min. Because the perfusion speed was slow, the exact time course of the D_2O effect could not be followed. The stretching device was made from electromagnetic vibrators (Ling Dynamic Systems) which produce linear displacements. The stretches of the muscle were recorded by displacement transducers (Hewlett-Packard, 7DCDT 250) which are attached to the shaft of the stretchers. The relaxed length of the muscle was 3.1 mm. Room temperature was 24°.

assumed (21) to be −25 mV. It was also assumed that D_2O did not change the reversal potential. In the five cells, the value of ΔG decreased to 36% (at 100 msec) and 30% (at 1 sec) of the control values in D_2O. Thus, our conclusion is that D_2O produces about 60–70% decrease in the magnitude of the transduction mechanism that links the deformation of the dendrites with the permeability increase.

We also studied effects of D_2O on the action potential of the stretch receptor neuron. The height of action potential did not change appreciably (in five cells the height was higher by 4% in D_2O than in H_2O), but the time course of the action potential became slower. Thus, the rate of rise of action potential in D_2O was 69% of the control, and the duration of the action potential (measured at the mid-height) was 145% of the control. These results on action potential agree well with those reported in the squid giant axon (6, 7).

It would be interesting to see whether D_2O has similar effects on receptor potentials of other kinds of sensory receptors. Thus, we might be able to infer the specificity and the general property of the transduction mechanisms of various kinds of receptor potentials. We do not know the mechanisms of D_2O action on the receptor potential. We can, nevertheless, speculate on several possibilities merely as working hypotheses: (*i*) the intracellular calcium ion concentration may be elevated by D_2O. It is possible that the calcium trapping mechanism of the intracellular organelles is altered by D_2O. It is known that an increase in the intracellular calcium ions reduces the sensitivity of the invertebrate photoreceptor (22). (*ii*) The microtubules, which are seen in abundance in the dendrites of the stretch receptor neuron (23), might be involved in the production of the receptor potential. D_2O is known to alter the structure and function of microtubules (24, 25). Moran and Varela (26) obtained some evidence that the microtubules may play a role in sensory transduction. (*iii*) There might exist chemical reactions, the product of which (analogous to the transmitter substance) has an ability to increase the permeability of the dendritic membrane. D_2O might affect some steps of these chemical reactions, leading to a decrease in the concentration of this product. (*iv*) The molecular configuration of the ionic channels in the dendrite membrane may be changed by D_2O.

It is hoped that further investigations will reveal the mechanism of the D_2O action on the receptor. It may lead to a better understanding of the transduction mechanism of the receptor potential, about which almost nothing is known.

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